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(71) Applicant (for all designated States except US): MUNIN
CORPORATION [US/US]; P.O. Box 3067, Oak Park, IL
60303-3067 (US).

(72) Inventor; and

(75) Inventor/Applicant (for US only): LAU, Lester, F.
[US/US]; 2677 N. Orchard Street, Chicago, IL 60614 (US).

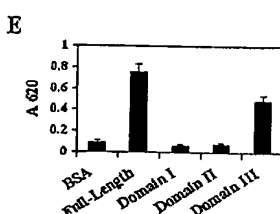
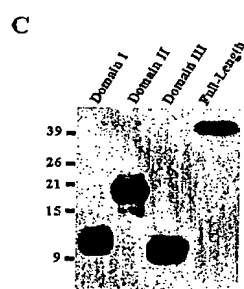
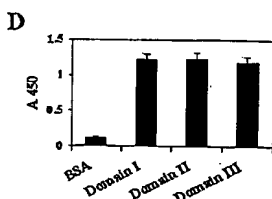
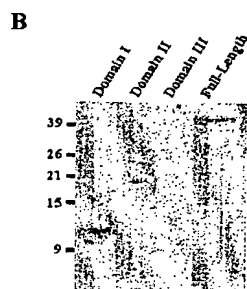
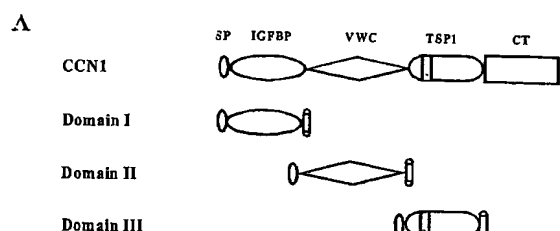
(74) Agents: CLOUGH, David, W. et al.; Howrey Simon
Arnold & White, LLP, Box No. 34, 1299 Pennsylvania
Avenue, N.W., Washington, DC 20004-2402 (US).

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(54) Title: CCN1 COMPOSITIONS AND METHODS



(57) Abstract: The angiogenic inducer CCN1 (cys-
teine-rich 61, CYR61), a secreted matricellular protein of the
CCN family, is a ligand of multiple integrins including $\alpha_6\beta_1$.
Previous studies have shown that CCN1 interaction with
integrin $\alpha_6\beta_1$ mediates adhesion of fibroblasts, endothelial
cells, and smooth muscle cells, as well as migration of
smooth muscle cells. Recently, we have reported that
CCN1-induced tubule formation of unactivated endothelial
cells is also mediated through integrin $\alpha_6\beta_1$. In this study, we
demonstrate that human skin fibroblasts adhere specifically
to the T1 sequence (GQKCIQTTSWSQCSKS) within
domain III of CCN1, and this process is blocked by anti- α_6
and anti- β_1 monoclonal antibodies. Alanine substitution
mutagenesis of the T1 sequence further defines the sequence
TTSWSQCSKS as the critical determinant for mediating
 $\alpha_6\beta_1$ -dependent adhesion. Soluble T1 peptide specifically
inhibits fibroblast adhesion to CCN1 in a dose-dependent
manner. Furthermore, T1 also inhibits cell adhesion to other
 $\alpha_6\beta_1$ ligands including CCN2 (CTGF), CCN3 (NOV), and
laminin, but not to ligands of other integrins. In addition,
T1 specifically inhibits $\alpha_6\beta_1$ -dependent tubule formation of
unactivated endothelial cells in a CCN1-containing collagen
gel matrix. To confirm that T1 binds integrin $\alpha_6\beta_1$ directly,
we perform affinity chromatography and show that integrin
 $\alpha_6\beta_1$ is isolated from an octylglucoside extract of fibroblasts
on T1-coupled Affi-gel. Taken together, these findings
define the T1 sequence in CCN1 as a novel binding motif
for integrin $\alpha_6\beta_1$, and form the basis for the development
of peptide mimetics to examine the functional role of $\alpha_6\beta_1$
in angiogenesis.



SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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